Studies on the Enzymatic Utilization of Amino Acyl Adenylates: the Formation of Adenosine Triphosphate*

PAUL BERG

Department of Microbiology, Washington University School of Medicine, St. Louis, Missouri

(Received for publication, March 3, 1958)

The enzymatic formation of acetyl coenzyme A from adenosine triphosphate (ATP),[1] acetate, and CoA was recently shown to occur (1, 2) by a two step reaction mechanism. This involves first, the reaction of ATP and acetate with an elimination of inorganic pyrophosphate and the formation of the 5′-phospho acetyl derivative of A5P. In the presence of CoA, this acetyl derivative is cleaved to form acetyl CoA and A5P (Equations 1 to 3).

$$\text{ATP} + \text{acetate} \overset{Mg^{++}}{\rightleftharpoons} \text{acetyl adenylate} + \text{PP} \quad (1)$$

$$\text{Acetyl adenylate} + \text{CoA} \rightleftharpoons \text{acetyl CoA} + \text{A5P} \quad (2)$$

$$\text{ATP} + \text{acetate} + \text{CoA} \overset{Mg^{++}}{\rightleftharpoons} \text{acetyl CoA} + \text{A5P} + \text{PP} \quad (3)$$

The widespread occurrence of this type of reaction in the activation of other fatty acids (3–6), inorganic acids (7–9), and other acyl compounds (10, 11) has been established by a number of investigators. Of particular interest was the discovery by Hoagland *et al.* (12, 13) and others (14–18) of enzymes which catalyze an amino acid-dependent exchange of ATP and PP^{32}. Purification of several of these enzymes and further studies of the mechanism of the reaction (14–17) revealed that they were relatively specific for a single amino acid and furthermore, that the reaction very likely involved the formation of a carboxyl phosphate anhydride derivative of A5P and the amino acid with the liberation of inorganic pyrophosphate (Equation 4).

$$\text{ATP} + \text{amino acid} \overset{Mg^{++}}{\rightleftharpoons} \text{amino acyl adenylate} + \text{PP} \quad (4)$$

The evidence supporting this hypothesis is as follows: (*a*) There is essentially no exchange of PP^{32} with ATP in the absence of the amino acid (15, 16); (*b*) in the presence of hydroxylamine there is an accumulation of equivalent amounts of the amino acid hydroxamate, A5P, and PP (15, 16); (*c*) free A5P is not formed as evidenced by the failure to find significant exchange of C^{14}-A5P with ATP (13, 15), and (*d*) O^{18} from the carboxyl group of the amino acid is transferred to the phosphate group of A5P in the presence of hydroxylamine (19, 20). Various attempts to isolate the postulated amino acyl adenylates have to date been unsuccessful and these failures have led to the notion that these compounds do not exist in the free state but rather in a complex with the enzyme (13).

* This work was supported by a grant from the U. S. Public Health Service.

[1] The abbreviations used are: ATP, adenosine triphosphate; CoA, coenzyme A; PP, inorganic pyrophosphate; A5P, adenosine-5′-monophosphate; Tris, tris(hydroxymethyl)aminomethane. A-2, alcohol fraction; AS-1, -2, ammonium sulfate fractions.

More direct evidence supporting the proposal of amino acid adenylate formation was obtained by using the chemically synthesized derivatives. DeMoss *et al.* (21) demonstrated that the enzyme from *Escherichia coli* which catalyzes the L-leucine-dependent exchange of PP^{32} with ATP also effects the conversion of L-leucyl adenylate to ATP. Subsequently it was reported (22) that L-methionyl adenylate is converted to ATP by an analogous enzyme isolated from yeast. In the present paper several aspects of the conversion of methionyl adenylate and other amino acyl adenylates to ATP are reported and in a second study (23) methods for the chemical synthesis and purification of a number of amino acyl adenylates are described.

MATERIALS AND METHODS

PP^{32} was prepared by heating $Na_2HP^{32}O_4$ at 400° for 1 hour and isolated by chromatography on a Dowex 1 Cl^- column. ATP, triphosphopyridine nucleotide, hexokinase, and glucose-6-phosphate dehydrogenase were purchased from the Sigma Chemical Company. All the amino acids used were Cfp grade as provided by the California Foundation for Biochemical Research. The various amino acid adenylates were prepared, purified, and determined as described in a second report (23).

Assay Procedures—Generally two methods were used to measure the formation of ATP from the amino acyl adenylates. The first relied on the conversion of PP^{32} to a Norit-adsorbable form (15, 24). The incubation mixture (1 ml.) contained 160 μmoles of potassium succinate buffer, pH 5.6, 5 μmoles of $MgCl_2$, 2 μmoles of PP^{32} containing 2.5 to 10 × 10^4 c.p.m. per μmole, between 1 and 1.5 μmoles of the amino acid derivative and the enzyme. All the components except the amino acyl adenylate were mixed, equilibrated at 37° for 3 minutes and then the amino acyl adenylate was added. After 3 minutes the reaction was terminated by the addition of 0.5 ml. of 0.7 M perchloric acid and the ATP formed was adsorbed on Norit and treated as previously described (15). 1 unit of activity is equal to the formation of 1 μmole of ATP in 15 minutes under these conditions. In the second procedure the same incubation mixture was used except for the substitution of unlabeled PP for the PP^{32} and the ATP formed was determined spectrophotometrically by TPN reduction in the coupled hexokinase, glucose-6-phosphate dehydrogenase system (25). The values obtained with these two procedures differed by less than 10 per cent. Using PP^{32} incorporation as a measure of the reaction, the amount of ATP formed was proportional to the amount of enzyme protein added. Thus with 3.3, 6.5, 13, 26, and 52 μg. of protein, there were 23, 24, 25, 25, and 26 units of activity per mg. of protein, respectively.

The purification of the enzyme was followed by measuring the L-methionine-dependent exchange of PP^{32} and ATP as previously described (15). The unit is defined as the incorporation of 1 μmole of PP^{32} into ATP per 15 minutes.

Enzyme Preparation—In a previous report (15), a procedure for the purification of an enzyme from brewers' yeast, which catalyzes the exchange of PP^{32} with ATP in the presence of L-methionine, was described. This involved fractionation of the yeast extract with alcohol followed by ammonium sulfate precipitation. These procedures yielded a preparation with a specific activity of about 15 (units per mg. of protein) compared to the initial extract which had a specific activity of approximately 0.2. In the present work, the purification procedure was extended with a view towards achieving more purified enzyme and to obtaining fractions with varying specific activities for studies of the specificity of the reaction. The following is a description of the preparative procedures for the enzyme fractions used in the current work.

To 25 ml. of the previously described fraction (15) AS-1 (specific activity 8.1, 4.9 mg. of protein per ml) was added 0.5 ml. of a solution of crystalline pancreatic ribonuclease (10 mg. per ml.) and the mixture was incubated at room temperature for 15 minutes. After cooling to 3°, 6.5 gm. of ammonium sulfate were added and the pH was adjusted to pH 4.6 by the addition of 0.6 ml. of 1 N sulfuric acid. After 5 minutes the precipitate was centrifuged and discarded. 2.1 gm. of ammonium sulfate were added to the supernatant fluid and after 5 minutes the precipitate was centrifuged and dissolved in 10 ml. of 0.1 M potassium succinate buffer, pH 6.0 (AS-2; specific activity 16.9; 2.5 mg. of protein per ml.).

To 4 ml. of fraction AS-2 were added 8 ml. of cold water and 1 ml. of aged alumina Cγ gel (26) (containing 15 mg. dry weight per ml.). The gel was centrifuged and washed once with 10 ml. of water, once with 10 ml. of 0.05 M potassium phosphate buffer, pH 6.5, and then with two 5 ml. portions of 0.1 M potassium phosphate buffer, pH 7.5. The major portion of the enzymatic activity appeared in the first pH 7.5 eluate (48 per cent) and an additional 10 per cent was present in the second pH 7.5 eluate (first Cγ gel eluate; specific activity 32; 0.5 mg. of protein per ml.).

Fraction AS-2 was also further purified by zone electrophoresis on a cellulose column (27). 2 ml. of AS-2 (specific activity 17.5; 2.7 mg. of protein per ml.), which originally had been dissolved in 0.02 M Tris buffer, pH 8.0, containing 0.02 M KCl and then dialyzed for 3 hours at 3° against the same buffer, was layered on top of a cellulose column (50 × 1 cm.) and subjected to a potential of 420 v. for 15 hours at 3°. The enzyme was recovered by displacement elution from the column with 0.02 M Tris buffer, pH 8.0, containing 0.02 M KCl. 50 per cent of the enzyme activity was eluted in 4 fractions (Fractions 11 to 14). The specific activities of the Fractions 11 to 14 were 33.5, 42.5, 32.6, and 20.0, respectively.

RESULTS

Upon examination of Equation 4, we may predict that an enzyme preparation catalyzing the L-methionine-dependent exchange of PP^{32} with ATP should convert L-methionyl adenylate to ATP in the presence of PP at a rate not slower than the rate of the exchange. Fig. 1 shows the rate of ATP synthesis from the chemically prepared L-methionyl adenylate. Calculation of the initial rate gives a figure of 15.9 μmoles of ATP formed per 15 minutes per mg. of protein. The rate for the exchange reaction under the identical conditions was 4.6 μmoles of PP^{32} incorporated into ATP per 15 minutes per mg. of protein. The conversion of L-methionyl adenylate to ATP did not go to completion but stopped when approximately 40 to 50 per cent of it appeared as ATP. This limited conversion was due to the destruction of the methionyl adenylate during the period of the reaction as indicated by the following experiment. An incubation of 0.91 μmole of methionyl adenylate and excess PP for 10 minutes resulted in the formation of 0.39 μmole of ATP, but a second 10 minute incubation with a fresh addition of enzyme or of PP resulted in no further increase in ATP (0.39 μmole of ATP). However, a second addition of the amino acyl adenylate after the first incubation, followed by a second 10 minute incubation period, yielded a total of 0.79 μmole of ATP as expected. Further discussion of the lability of methionyl adenylate is presented later.

The requirements for the conversion of methionyl adenylate to ATP are shown in Table I. In the absence of enzyme, PP, Mg^{++}, or methionyl adenylate, there was no ATP formed. Furthermore, if the methionyl adenylate was treated with 0.01 N KOH for 5 minutes at 25° to form free A5P and L-methionine, there was no ATP formation.

Stoichiometry of the Reaction—Attempts to study the stoichiometry of the reaction and obtain a balance of the various components were complicated by the marked instability of the substrate under the conditions used. Measurements of the L-methionyl adenylate disappearance were therefore made in the presence and absence of PP to determine the amount of methionyl adenylate utilized for ATP formation. The results, shown in Table II, indicate that the increment in methionyl adenylate disappearance resulting from the presence of PP is in agreement with the amount of ATP synthesized and with the amount of PP^{32} incorporated into ATP.

It is not yet clear whether the methionyl adenylate breakdown in the absence of PP is enzymatically catalyzed. We have found that the half-life of methionyl adenylate under the condi-

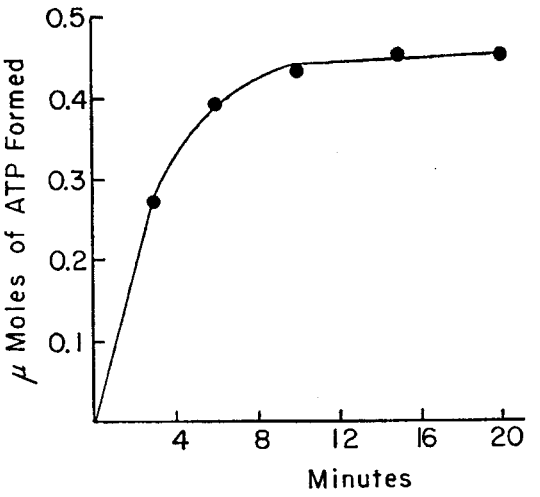

FIG. 1. Kinetics of conversion of L-methionyl adenylate to ATP: The reaction mixture contained, in 1 ml., 160 μmoles of potassium succinate buffer, pH 5.6, 5 μmoles $MgCl_2$, 2 μmoles PP^{32} containing 4.6×10^4 c.p.m. per μmole, 1.05 μmoles L-methionyl adenylate, 85 μg. of enzyme AS-1, specific activity 7.8. Temperature, 37°.

TABLE I

Requirements for L-methionyl adenylate conversion to ATP

Components	ATP formed*
	μmoles
Complete	0.40
Minus PP	0.00
Minus L-methionyl adenylate	0.00
Minus $MgCl_2$	0.04
Minus enzyme	0.00
Complete, but with hydrolyzed L-methionyl adenylate	0.00

* ATP was determined in a 0.1 ml. aliquot of the heated reaction mixture with the coupled hexokinase, glucose-6-phosphate dehydrogenase assay (25).

The reaction mixture contained, in 1 ml., 150 μmoles potassium succinate buffer, pH 5.5, 5 μmoles of $MgCl_2$, 3 μmoles of PP, 1.6 μmoles of methionyl adenylate, 165 μg. of AS-1, specific activity 5.0. Temperature 37°, time 15 minutes. The L-methionyl adenylate was hydrolyzed in 0.01 N KOH at 25° for 5 minutes and then the solution was neutralized with HCl.

TABLE II

The disappearance of L-methionyl adenylate and formation of ATP

Time	L-Methionyl adenylate*	ATP formed†	PP^{32} incorporated into ATP‡
minutes	*μmole*	*μmole*	*μmole*
5	−0.12	0.14	0.13
10	−0.23	0.22	0.21
15	−0.26	0.25	0.24

* L-Methionyl adenylate was determined as the hydroxamate with acid $FeCl_3$ (23). The values for L-methionyl adenylate disappearance in the presence of PP were 0.60, 0.86, and 0.96 μmole, while in the absence of PP the values were 0.48, 0.63, and 0.70 μmole.

† ATP was measured in a heated aliquot of the reaction mixture with hexokinase and glucose-6-phosphate dehydrogenase (25).

‡ PP^{32} incorporation was determined as described in "Methods."

The reaction mixture contained, in 0.5 ml., 75 μmoles of potassium succinate buffer, pH 5.6, 2.5 μmoles of $MgCl_2$, 2 μmoles of PP or PP^{32} containing 5.4×10^4 c.p.m. per μmole, 1.15 μmoles of L-methionyl adenylate and 28 μg. of AS-1, specific activity, 7.3. Temperature, 37°.

tions shown in Table II (absence of PP) is 7.5 minutes, whereas with a heat-inactivated sample of the enzyme it was about 14 minutes. This is to be contrasted with a value of greater than 25 minutes observed in the absence of the enzyme preparation. This 2-fold increase in the rate of breakdown of amino acyl adenylate may be due to enzymatic activity or to some other heat-labile component present in the enzyme preparation. Further studies are needed to clarify this point.

In order to equate the conversion of methionyl adenylate to ATP with the methionine-dependent ATP-PP^{32} exchange reaction, it seemed pertinent to determine if both reactions are catalyzed by the same enzyme. A number of fractions at various stages of purification have been examined for both activities and within the limits of the accuracy of the measurements made, the ratios of the two activities were essentially constant (Table III). It should be pointed out that these ratios compare the two reactions under somewhat different conditions. The exchange reaction is carried out at pH 8.0 whereas the amino acyl adenylate conversion to ATP is performed at pH 5.6. The exchange reaction activity at 5.6 is about 60 per cent that of pH 8.0 and therefore the ratio is actually between 0.25 and 0.33. No preparation has been found which catalyzes one reaction and not the other. As another line of evidence, experiments which will be discussed later showed that free L-methionine competitively inhibits the conversion of L-methionyl adenylate to ATP and that the K_I value for methionine is in close agreement with the observed K_s value for methionine in the ATP-PP^{32} exchange assay. These findings suggest that L-methionine acts at the same site and presumably with the same enzyme when functioning as a substrate or as an inhibitor.

Specificity—It was previously reported (15) that the L-methionine-activating enzyme was relatively specific for L-methionine since other naturally occurring amino acids did not promote a significant ATP-PP^{32} exchange reaction. It was therefore of interest to examine the specificity with respect to the reverse reaction, namely, the conversion of other amino acyl adenylates to ATP (Table IV). Although ATP formation from L-methionyl adenylate is most rapid, there is significant ATP formation from L-seryl adenylate, L-phenylalanyl adenylate, and even D-methionyl adenylate, but little or no utilization of L-tryptophanyl adenylate. ATP formation was confirmed by the spectrophometric assay and was not observed when the amino acyl adenylates had been hydrolyzed with dilute alkali. Under the identical conditions however, there was very little or no significant exchange of PP^{32} with ATP in the presence of L-serine, L-phenylalanine, L-tryptophan, or D-methionine (Table V). These measurements of the exchange reaction, now made at pH 5.6, are in agreement with the previous findings carried out at pH 8.0.

The finding of ATP formation from D-methionyl adenylate posed the question of whether there was contamination of the material with the L-methionine derivative. The L-methionine

TABLE III

Comparison of the rate of the ATP-PP^{32} exchange reaction with the rate of conversion of L-methionyl adenylate to ATP in various enzyme preparations

Fraction	Specific activity, ATP-PP^{32} exchange reaction *A*	Specific activity, L-methionyl adenylate conversion to ATP *B*	Ratio, *A*:*B*
	units/mg. of protein	*units/mg. of protein*	
Alcohol (A-2)	2.4	5.1	0.47
Ammonium sulfate (AS-1)	7.3	16.0	0.46
Ammonium sulfate (AS-2)	13.1	25.8	0.51
Alumina C_γ gel eluate	32.0	65.1	0.49
Cellulose column electrophoresis of AS-2, Fraction:			
10	10.4	20.8	0.50
11	33.5	60.5	0.55
12	42.5	86.7	0.49
13	32.6	65.0	0.50
14	20.0	40.7	0.49
15	9.7	20.6	0.47

TABLE IV

The conversion of amino acyl adenylates to ATP by the methionine-activating enzyme

Amino acyl adenylate	ATP formation
	μmoles/15 min./mg. of protein
None	0.05
L-Methionyl adenylate	42.0
L-Seryl adenylate	10.0
L-Phenylalanyl adenylate	5.0
L-Tryptophanyl adenylate	0.10
D-Methionyl adenylate	6.0

The reaction mixture contained, in 1 ml., 150 μmoles of potassium succinate buffer, pH 5.6, 5 μmoles of $MgCl_2$, 2 μmoles of PP^{32} containing 10^5 c.p.m. per μmole, 1.1 μmoles of L-methionyl adenylate, 1.2 μmoles of L-seryl adenylate, 1.2 μmoles of L-phenylalanyl adenylate, 1.2 μmoles of L-tryptophanyl adenylate, and 1.1 μmoles of D-methionyl adenylate. In all cases, except with L-tryptophanyl adenylate, enough enzyme was used so that 0.25 to 0.50 μmole of ATP was formed during the incubation period.

TABLE V

The exchange of PP^{32} and ATP with L-methionine, L-serine, L-phenylalanine, L-tryptophan, and D-methionine

Amino acid	PP^{32} incorporated into ATP
	units/mg. of protein
None	<0.10
L-Methionine	10.4
L-Serine	<0.10
L-Phenylalanine	<0.10
L-Tryptophan	<0.10
D-Methionine	<0.10

The reaction mixture was the same as described in Table IV except that 2 μmoles of ATP and 2 μmoles of each of the amino acids replaced the amino acid adenylates.

could have been present as an original contaminant in the D-methionine; it could have also been formed during the synthesis of the adenylate or during the incubation with the enzyme. All of these possibilities were eliminated by the following experiments. Polarimetric examination of the starting D-methionine showed an $[\alpha]_D$ in 5 N HCl of +23.9° compared to the value +23.4° given in the literature (28). Moreover, the sample of D-methionine did not catalyze the exchange of ATP and PP^{32} at a level which would have detected a 1 per cent contamination with L-methionine. Hydrolysis of D-methionyl adenylate with 0.01 N KOH and testing of the hydrolysate for its ability to replace L-methionine in the exchange reaction was likewise negative. For example, in one experiment, 1 μmole of L-methionine and a hydrolysate of L-methionyl adenylate containing 1 μmole of L-methionine gave an incorporation of 0.2 and 0.19 μmole of PP^{32}, respectively, into ATP. On the other hand, 1 μmole of D-methionine, free or in the form of the D-methionyl adenylate hydrolysate, gave less than 1 per cent of this value. Furthermore, preincubation of the D-methionyl adenylate with the enzyme and then followed by hydrolysis and testing in the exchange reaction likewise gave no evidence for the presence of L-methionine.

This apparently anomalous finding posed the question whether additional mechanisms exist for utilizing the amino acid adenylates other than that shown in Equation 4. We investigated this question first by determining if the conversion of L-methionyl adenylate and other amino acid adenylates to ATP was catalyzed by the same enzyme. Various enzyme preparations were compared for their ability to convert L-methionyl and L-seryl adenylates to ATP (Table VI). The results showed that the ratios of the two activities were essentially constant over an approximately 17-fold range of enzyme purification. Moreover, attempts to effect a preferential heat inactivation of one activity with respect to the other demonstrated that the kinetics of inactivation were essentially first order with both substrates and that the ratio of the two activities remained the same throughout (Table VII). The same result was obtained under somewhat varied conditions for the heat inactivation (Experiment 2).

Additional evidence which supports the idea that a single enzyme catalyzes the conversion of both L-methionyl and L-seryl adenylates to ATP was obtained by kinetic experiments. L-Methionine competitively inhibits the conversion of both L-methionyl and L-seryl adenylates to ATP (Table VIII). L-Serine (2 to 48 × 10^{-3} M), on the other hand, does not inhibit ATP formation from either of these substrates. Dixon (29) has described a graphic method for determining the K_I and K_s values which involves a plot of $1/V$ against the inhibitor concentration (I) at two different substrate concentrations (Fig. 2*A* and *B*). The point at which the two lines intercept one another is equal to $-K_I$ and the intercept of each curve with the abscissa is equal to $-K_I(S/K + 1)$. From these data, the K_I values for L-methionine acting as an inhibitor of ATP formation from L-methionyl and L-seryl adenylates were 3

TABLE VI

The activity of various enzyme preparations in the conversion of L-methionyl and L-seryl adenylates to ATP

Preparation	Specific activity		Ratio, *A*:*B*
	L-Methionyl adenylate conversion to ATP *A*	L-Seryl adenylate conversion to ATP *B*	
	units/mg. of protein	*units/mg. of protein*	
A-2	5.1	1.2	4.3
AS-1	16.0	4.0	4.0
AS-2	25.8	6.1	4.2
AS-2a	33.4	8.1	4.1
AS-2b	27.2	6.6	4.1
AS-2c	25.9	6.5	4.0
AS-2d	19.8	4.8	4.1
Alumina C_γ gel eluate	65.1	15.9	4.1
Cellulose column electrophoresis of AS-2, Fraction:			
10	20.8	4.1	5.1
11	60.5	12.6	4.8
12	86.7	19.9	4.4
13	65.0	15.4	4.2
14	40.7	8.4	4.9
15	20.6	4.0	5.1

AS-2a to 2d refers to ammonium sulfate fractions derived from AS-2 and which were prepared as follows: 0 to 0.44 saturation (AS-2a), 0.44 to 0.48 saturation (AS-2b), 0.48 to 0.51 saturation (AS-2c), 0.51 to 0.62 saturation (AS-2d).

and 3.5×10^{-4} M, respectively. The K_s for L-methionine in the exchange reaction measured under the same conditions (pH 5.6) was 2.6×10^{-4} M. The agreement between the apparent dissociation constants of free methionine acting both as a substrate and inhibitor suggests that it is acting at the same site in both reactions. The K_s for L-methionine at pH 5.6 was as mentioned above 2.6×10^{-4} M but calculation of previous data obtained at pH 8.0 gave a value of 1.0×10^{-5} M or about 25 times lower. The factors contributing to this higher affinity at pH 8.0 are not known and require further work. The K_s for L-methionyl adenylate determined graphically (29) was $3.7 \pm 0.8 \times 10^{-5}$ M and the K_s for L-seryl adenylate was $1.2 \pm 0.2 \times 10^{-3}$ M. Thus there seems to be about a 30-fold difference in the apparent affinity of the two amino acid adenylates at pH 5.6.

During the course of these studies Dr. W. P. Jencks suggested[2] that amino acids other than L-methionine might be active in the ATP-PP^{32} reaction if tested at high concentrations. When this was carried out it was found that L-serine, D-methionine, and

[2] Private communication.

TABLE VII

Heat inactivation of enzyme preparation catalyzing conversion of L-methionyl and L-seryl adenylates to ATP

Experiment	Time at 45°	L-Methionyl adenylate conversion to ATP *A*	L-Seryl adenylate conversion to ATP *B*	Ratio, *A*:*B*
	min.	*units/ml.*	*units/ml.*	
I	0	7.6	1.8	4.2
	1	6.4	1.6	4.0
	2	3.6	0.88	4.1
	3	2.3	0.52	4.4
	5	1.2	0.28	4.3
	7	0.68	0.16	4.3
II	0	97	23	4.2
	2.5	92	18	5.1
	5	80	18	4.4
	7.5	68	16	4.2

In Experiment I the enzyme (AS-2, 280 μg. per ml.) was kept in a water bath at 45° and aliquots were removed at various times, cooled and the activity with both L-methionyl and L-seryl adenylates determined. In Experiment II, the enzyme (AS-2, 3.1 mg. per ml.) containing 1.8 μmoles of ATP and 2 μmoles of $MgCl_2$ per ml. was treated as above. The cooled aliquots were diluted 50- to 200-fold in the final reaction mixture so that the amount of ATP present was negligible.

TABLE VIII

The effect of L-methionine on the conversion of L-methionyl and L-seryl adenylates to ATP

Concentration of amino acyl adenylate	Concentration of L-methionine	Amino acyl adenylate conversion to ATP	Inhibition
× 10^3 M	× 10^3 M	*units/mg. of protein*	%
L-Methionyl adenylate:			
1.1	0.0	31.5	
1.1	2.4	24.0	24
1.1	5.4	19.5	38
1.1	10	13.6	57
1.1	20	8.3	74
0.55	10	9.2	71
2.2	11	18.0	43
2.2	21	11.8	62
L-Seryl adenylate:			
1.2		7.4	
1.2	0.6	3.5	53
1.2	1.2	2.5	66
2.4	0.6	4.4	40
2.4	1.2	3.5	53
4.8	0.6	5.3	28
4.8	1.2	4.3	42

The assay conditions are described in "Methods." The enzyme used was AS-2 with a specific activity of 16.

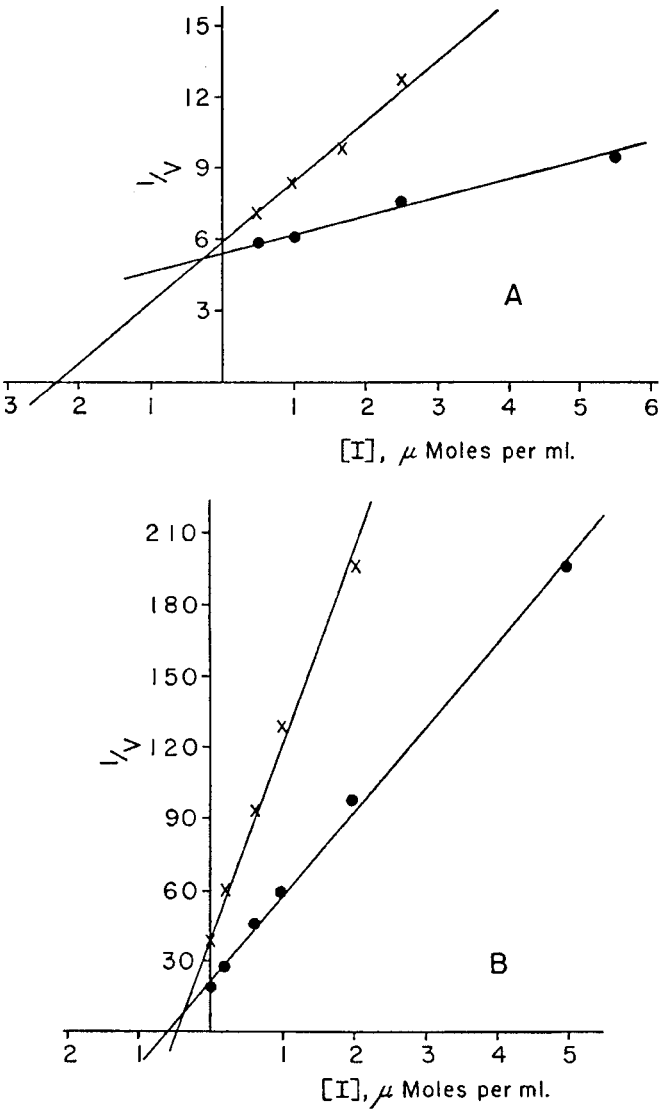

FIG. 2. Analysis of the inhibition of ATP formation from L-methionyl and L-seryl adenylates by L-methionine: *Curve A*, inhibition of L-methionyl adenylate conversion to ATP; *Curve B*, inhibition of L-seryl adenylate conversion to ATP. $1/V$ is the reciprocal of the rate of ATP formation in μmoles per minute per mg. of protein. The reactions were carried out as described in "Methods."

L-threonine, had low but significant activity in promoting the exchange, while L-tryptophan (0.03 M) and L-isoleucine (0.14 M) had no detectable activity. Fig. 3 shows that at 0.13 M, L-serine does promote a slow incorporation of PP^{32} into ATP. Higher concentrations of L-serine were inhibitory. D-Methionine (0.03 M) and L-threonine (0.25 M) were one-fifth and two-thirds as active as L-serine while higher concentrations were also inhibitory. Since essentially all the studies of the amino acid-activating enzymes (15–17) have revealed a very high affinity of the enzyme for the amino acid in question, its seems unlikely that this slow rate of exchange found at high concentrations of amino acid is due to the presence of small amounts of enzymes specific for these amino acids. Rather, it seems more likely that the enzyme which is "specific" for L-methionine has a relatively low affinity and activity with certain other amino acids. These findings point to the likelihood that although the enzyme is relatively specific for a single amino acid and its adenylate, it also utilizes other amino acids and their adenyl derivatives with a lower efficiency.

Similar findings have been reported with a fatty acid-activating enzyme by Jencks and Lipmann (6). They showed that with the enzyme specific for fatty acids of intermediate chain length (4 to 12 carbon atoms), and under their usual assay conditions, acetate (5×10^{-3} M) was not converted to acetyl CoA. However, acetyl adenylate was utilized for acyl CoA synthesis at about half the rate of hexanoyl adenylate. Further studies revealed that with higher concentrations of acetate, acetyl CoA synthesis was demonstrable; this being maximal at an acetate concentration of 0.4 M.

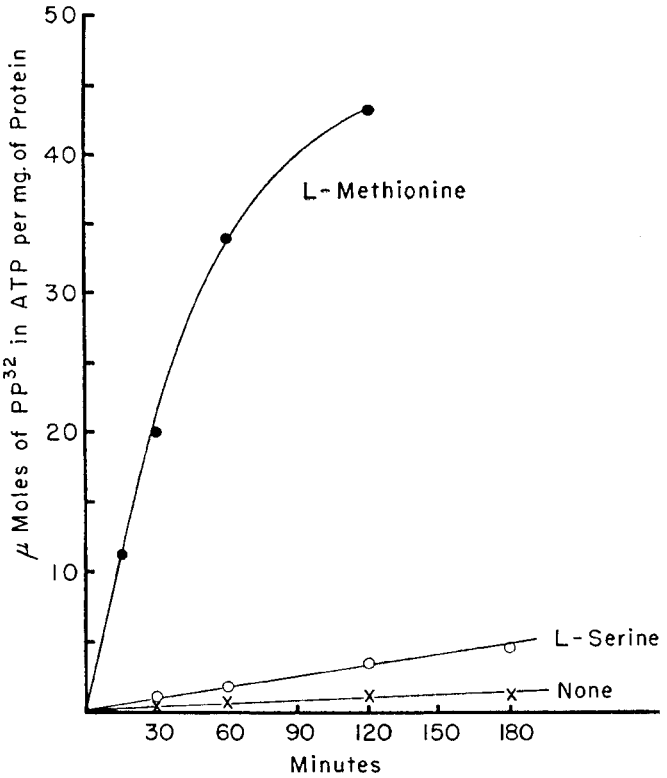

FIG. 3. The exchange of PP^{32} and ATP in the presence of high concentrations of L-serine: The reaction mixture (1 ml.) contained 100 μmoles of Tris buffer, pH 8.0, 5 μmoles of $MgCl_2$, 2 μmoles of ATP, 2 μmoles of PP^{32} containing 6×10^4 c.p.m. per μmole, 1 μmole of L-methionine or 125 μmoles of L-serine. Temperature, 37°.

DISCUSSION

The conversion of L-methionyl adenylate to ATP by the purified methionine-activating enzyme supports the mechanism proposed for the L-methionine dependent exchange of ATP and PP^{32} shown in Equation 4. The surprising finding in these experiments was that, although the enzyme is relatively specific for L-methionine in the exchange reaction, other amino acid adenylates are also converted to ATP. Purification of the enzyme and kinetic studies have indicated however, that at least in the case of the utilization of L-methionyl and L-seryl adenylates, the same enzyme is responsible for both reactions. Further studies revealed that the specificity for methionine was relative since at very high concentrations of L-serine, L-threonine, and D-methionine there was a detectable ATP-PP^{32} exchange. Similar findings to those described here have also been reported for experiments with crude extracts of *Escherichia coli* by DeMoss *et al.* (21). These workers observed that L-alanyl adenylate was converted to ATP while L-alanine was inactive in promoting the exchange of PP^{32} and ATP. We have made similar findings in *E. coli* extracts with L-seryl and L-threonyl adenylates.[3] Exactly which enzyme (or enzymes) is responsible for the utilization of these compounds remains to be determined.

The failure to detect the accumulation of L-methionyl adenylate from ATP and L-methionine remains a puzzling feature of this reaction. Because of the rapid destruction of methionyl adenylate at pH 8 it does not seem likely that the enzymatically formed compound exists to any significant extent in the free state. If this were so, one might expect to observe a significant increase in the breakdown of ATP to A5P and PP in the presence of L-methionine. This, however, was not observed in our earlier studies (15). The question of whether the enzymatically formed amino acid adenylates are bound to the enzyme and thereby stabilized remains to be resolved.

The mechanism of methionyl adenylate formation resembles the first step in the formation of the fatty acid-CoA derivatives (2–6). More recent studies (30) indicate that the similarity extends to the second step as well since the methionine-activating enzyme has been shown to transfer the amino acid moiety to a polyribonucleotide. Thus the amino acid-activating enzymes like the fatty acid-activating enzymes appear to catalyze both the transfer of an adenyl group from ATP to an acyl group and the transfer of the acyl group to an appropriate acceptor.

SUMMARY

A purified L-methionine-activating enzyme from yeast has been shown to convert L-methionyl adenylate to adenosine triphosphate (ATP) in the presence of inorganic pyrophosphate (PP). The rate of this conversion is consistent with its role as an intermediate in the L-methionine dependent exchange of PP^{32} and ATP.

ATP + L-methionine $\rightleftharpoons$ L-methionyl adenylate + PP

The same preparation also catalyzes a significant conversion of L-seryl, L-phenylalanyl, and D-methionyl adenylates to ATP even though it does not utilize the free amino acids in the exchange re-

[3] Unpublished observations of the author.

action. Purification studies have indicated that the enzyme which utilizes L-seryl adenylate is the same one which converts L-methionyl adenylate to ATP. This was further supported by the finding that the K_s for L-methionine in the exchange reaction (2.6×10^{-4} M) is the same as the K_I value for L-methionine acting as an inhibitor of L-methionyl adenylate conversion to ATP (3×10^{-4} M) and for L-seryl adenylate conversion to ATP (3.5×10^{-4} M).

REFERENCES

1. Berg, P., *J. Am. Chem. Soc.*, **77**, 3163 (1955).
2. Berg, P., *J. Biol. Chem.*, **222**, 991 (1956).
3. Talbert, P. T., and Huennekins, F. M., *J. Am. Chem. Soc.*, **78**, 4671 (1956).
4. Peng, C. H. L., *Biochim. et Biophys. Acta*, **22**, 42 (1956).
5. Moyed, H. S., and Lipmann, F., *J. Bacteriol.*, **73**, 117, (1957).
6. Jencks, W. P., and Lipmann, F., *J. Biol. Chem.*, **225**, 207 (1957).
7. Robbins, P. W., and Lipmann, F., *J. Am. Chem. Soc.*, **78**, 6409 (1956); *J. Biol. Chem.*, **229**, 837 (1957).
8. Bandurski, R. S., Wilson, L. G., and Squires, C. L., *J. Am. Chem. Soc.*, **78**, 6408 (1956).
9. Bachhawat, B. K., Woessner, J. F., Jr., and Coon, M. J., *Federation Proc.*, **15**, 214 (1956).
10. Maas, W., *3rd International Congress of Biochemistry, Brussels, Reports*, 1955.
11. Reed, L. J., Leach, F. R., Koike, M., and Levitch, M. E., *Federation Proc.*, **16**, 236 (1957).
12. Hoagland, M. B., *Biochim. et Biophys. Acta*, **16**, 288 (1955).
13. Hoagland, M. B., Keller, E. B., and Zamecnik, P. C., *J. Biol. Chem.*, **218**, 345 (1956).
14. DeMoss, J. A., and Novelli, G. D., *Biochim. et Biophys. Acta*, **22**, 49 (1956).
15. Berg, P., *J. Biol. Chem.*, **222**, 1025 (1956).
16. Davie, E. W., Koningsberger, V. V., and Lipmann, F., *Arch Biochem. Biophys.*, **65**, 21 (1956).
17. Schweet, R., *Federation Proc.*, **16**, 244 (1957).
18. Webster, G. C., *J. Biol. Chem.*, **229**, 535 (1957).
19. Hoagland, M. B., Zamecnik, P. C., Sharon, N., Lipmann, F., Stulberg, M. P., and Boyer, P. D., *Biochim. et Biophys. Acta*, **26**, 215 (1957).
20. Bernlohr, R., and Webster, G. C., *Arch. Biochem. Biophys.*, **73**, 276 (1958).
21. DeMoss, J. A., Genuth, S. M., and Novelli, G. D., *Proc. Natl. Acad. Sci.*, **42**, 325 (1956).
22. Berg, P., *Federation Proc.*, **16**, 152 (1957).
23. Berg, P., *J. Biol. Chem.*, **233**, 608 (1958).
24. Crane, R. K., and Lipmann, F., *J. Biol. Chem.*, **201**, 235 (1953).
25. Kornberg, A., *J. Biol. Chem.*, **182**, 779 (1950).
26. Willstätter, R. and Kraut, A., *Ber. deut. chem. Ges.*, **56**, 1117 (1923).
27. Porath, J., *Biochim. et Biophys. Acta*, **22**, 151 (1956).
28. Howe, E. E., In D. M. Greenberg (Editor), *Amino acids and proteins*, Charles C Thomas, Springfield, Ill., 1951.
29. Dixon, M., *Biochem. J.*, **55**, 170 (1953).
30. Berg, P., and Ofengand, E. J., *Proc. Natl. Acad. Sci.*, *44*, 78 (1958).